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Antibacterial and antioxidant activity of red melinjo (*Gnetum gnemon* L.) peel ethanolic extract

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A B S T R A C T

Red melinjo (*Gnetum gnemon* L.) peel is an underutilized by-product with potential as a natural antimicrobial and antioxidant source due to its content of phenolics, flavonoids, vitamin C, and carotenoids. This study evaluated the effective concentrations required to inhibit microorganisms representing Gram-positive bacteria (*Bacillus cereus*), Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), and molds (*Rhizopus oligosporus*), and the stability of antioxidant activity and key antioxidant-related components during short-term storage (0–12 days). Red melinjo peel was extracted using ethanol via maceration. Antimicrobial activity was assessed using the well diffusion method at extract concentrations of 0–16%, followed by determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for bacterial targets. Antioxidant activity was monitored using the DPPH assay (IC_{50}), alongside quantification of total phenolics, total flavonoids, vitamin C, and total carotenoids, including correlation analysis with antioxidant activity. The extract showed antibacterial activity against *B. cereus*, *P. aeruginosa*, and *E. coli*, with MIC values of 0.46%, 0.50%, and 0.56% and MBC values of 1.83%, 2.01%, and 2.22%, respectively, while no inhibitory effect was observed against *R. oligosporus*. A 12% extract concentration was selected as an effective level based on inhibition performance. Antioxidant activity was highest at day 0 (IC_{50} = 702.99 mg/L) and declined progressively with storage time, accompanied by reductions in phenolics, flavonoids, vitamin C, and carotenoids, indicating degradation of antioxidant contributors during storage. Overall, the findings support red melinjo peel ethanolic extract as a promising dual-function bioactive material and provide evidence that storage duration reduces antioxidant effectiveness through compositional changes in key bioactive compounds.

Keywords: Antimicrobial Activity; Antioxidant Activity; *Gnetum gnemon* L.; Red Melinjo Peel

1. INTRODUCTION

Melinjo (*Gnetum gnemon* L.) is indigenous to Southeast Asia and is widely distributed throughout many regions of Indonesia (Tani *et al.*, 2020). It belongs to the group of gymnosperms (Gymnospermae) and originates from tropical Asia, Melanesia, and the western Pacific. This plant holds significant economic value, as nearly all of its parts are useful for human consumption and industry. Its leaves, flowers, and mature fruit peels are commonly used as vegetables, while mature seeds are processed into traditional snacks like *emping*. The bark can be used to make rope, and the wood is utilized in the paper industry (Suryani & Zulkarnain, 2021). According to Badan Pusat Statistik (2015), Indonesia produced around 213,025 tons of melinjo in 2015. However, often the peel was discarded, indicating an untapped potential for further application in the food sector (Tani *et al.*, 2020; Parhusip & Sitanggang, 2011).

The melinjo fruit used in this research was ripe fruit with a red peel, which is known to contain high levels of phenolics, β-carotene, lycopene, carotenoids, vitamin C, tannins, saponins, and flavonoids. These bioactive compounds are found in greater amounts compared to unripe yellow or green melinjo peel and have been widely reported to possess antimicrobial and antioxidant potential (Octavia, 2010; Parhusip and Sitanggang, 2011; Ibrahim *et al.*, 2024). Such findings indicate that red melinjo peel represents a promising natural source of functional compounds that may be utilized for food preservation or medicinal applications.

Previous studies have demonstrated that red melinjo peel extract exhibits antibacterial activity, particularly when extracted using ethanol. Parhusip and Sitanggang (2011) reported that ethanol-based extracts showed inhibitory effects against certain pathogenic bacteria, highlighting the strong antimicrobial potential of phenolic and flavonoid constituents in the peel. However, their study did not clearly determine the minimum concentration

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required to effectively inhibit foodborne pathogens. Additionally, while antioxidant components in melinjo peel—such as phenolics, flavonoids, carotenoids, and vitamin C—are known to contribute significantly to its bioactivity, information regarding their stability during storage remains limited. Antioxidant degradation has been shown to occur over time, even under low-temperature conditions, due to the natural breakdown of sensitive bioactive molecules (Dewi *et al.*, 2022).

Despite these findings, research exploring the antimicrobial effectiveness of red melinjo peel extract against a broader range of microorganisms, including both Gram-positive and Gram-negative bacteria as well as molds, is still lacking. Moreover, the influence of storage duration on the antioxidant stability of the ethanolic extract has not been comprehensively studied. These gaps highlight the need for further investigation into the antimicrobial activity of red melinjo peel ethanolic extract against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Rhizopus oligosporus*, along with an assessment of how storage time affects its antioxidant properties and the key bioactive compounds responsible for these activities.

2. Material and Methods

2.1 Study area

This study was conducted in the Food Microbiology and Chemistry Laboratory, Department of Food Science and Technology, using laboratory-scale experimental procedures. Red melinjo (*Gnetum gnemon* L.) fruits were collected from Bogor, Indonesia. All microbiological and chemical analyses were carried out under aseptic conditions using standard laboratory equipment and procedures.

2.2 Materials

The materials used in this study included red melinjo (*Gnetum gnemon* L.) fruit peel, ethanol (pro analysis), industrial-grade ethanol, aquadest, and pure cultures of *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, and *Rhizopus oligosporus* ATCC 22959.

Other reagents used were crystal violet, iodine, acetone–alcohol solution (95% alcohol), safranin, immersion oil, Whatman no. 1 filter paper, Nutrient Agar (NA), Nutrient Broth (NB), Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), Plate Count Agar (PCA), DPPH, Folin–Ciocalteu reagent, Na_2CO_3, gallic acid, aluminium chloride ($AlCl_3$), quercetin, ascorbic acid, petroleum ether (pro analysis), chloroform, ammonia, concentrated H_2SO_4, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, HCl 2 N, $FeCl_3$ 1%, NaOH 10%, ether, anhydrous acetic acid, and sulfuric acid.

The equipment used in this research included a cabinet dryer, autoclave (Hirayama), laminar air flow (Esco), microscope, incubator (Memmert), analytical balance, refrigerator, oven (Memmert), desiccator (Duran), UV–Vis spectrophotometer, quartz cuvette, shaker, rotary evaporator (Buchi), colony counter (Funke Gerber), 35-mesh sieve (Tyler), blender (Philips), vacuum pump (Buchner), vortex, water bath, heater (Torrey Pines), magnetic stirrer, inoculating loop, evaporating dish, petri dishes (Gosselin), micropipette and tips, boiling flask (Duran or Iwaki Pyrex), thermometer, hair dryer (Philips), and Liquid Chromatography–Mass Spectrometry (LC-MS) instrument.

2.3 Preliminary stage

The red melinjo fruit peel was processed into powder and extracted following the method of Parhusip and Sitanggang (2011) with modifications. Fresh red melinjo fruits were washed, peeled, and dried in a cabinet dryer at 50 °C for 24 hours. The dried peels were blended and sieved using a 35-mesh sieve to obtain a fine powder. For extraction, the sample was macerated in ethanol (1:4 w/v) at room temperature for 24 hours with continuous shaking at 110 rpm. The resulting mixture was vacuum filtered through Whatman No. 1 paper, and the collected filtrate was concentrated with a rotary evaporator and further dried under nitrogen gas. The completed ethanolic extract was stored at 4–5 °C in a dark glass container for subsequent testing.

2.4 Antimicrobial activity test

The antimicrobial activity of the red melinjo peel ethanolic extract was tested using the agar well diffusion method described by Parhusip and Sitanggang (2011) with slight modifications. Four microbial species (*B. cereus*, *P. aeruginosa*, *E. coli*, and *R. oligosporus*) were used as test organisms. Extract concentrations of 0%, 4%, 8%, 12%, and 16% (w/v) were prepared. Each well on the agar plates was filled with the corresponding extract concentration. Plates containing bacteria were incubated for 24 hours at 37 °C, while plates containing molds were incubated for 48 hours at 30 °C. The diameters of the inhibition zones were measured with a vernier caliper, and these measurements were used to calculate the MIC and MBC based on the method outlined by Bloomfield (1991).

2.5 Antioxidant activity stability upon storage time

The antioxidant stability of the red melinjo peel ethanolic extract was evaluated according to the methods of Dewi *et al.* (2022) and Ibrahim *et al.* (2024), with modifications. The extract was stored in a dark bottle at 4–5 °C for 12 days. Antioxidant activity, total phenolic content, total flavonoid content, vitamin C, and carotenoid levels were analyzed on days 0, 3, 6, 9, and 12 of storage to determine changes in antioxidant properties over time.

2.6 Experimental design

The antimicrobial activity experiment used a completely randomized two-factorial design with two replications. The first factor was the type of microorganism (*B. cereus*, *P. aeruginosa*, *E. coli*, and *R. oligosporus*), and the second factor was the extract concentration (0%, 4%, 8%, 12%, and 16%).

The antioxidant stability test used a completely randomized one-factor design with five replications, where the factor was the storage time (days 0, 3, 6, 9, and 12).

2.7 Analytical methods

The following analyses were conducted: moisture content (AOAC, 2005), qualitative phytochemical screening (Asmara, 2017; Sassie, 2013; Simaremare *et al*, 2020; Khasanah *et al.*, 2020), antioxidant activity (Cahyana *et al.*, 2017; Shukla and Kushwaha, 2024), total phenolic content (Rao *et al.*, 2016), total flavonoid content (Cahyana *et al.*, 2017; Sembiring *et al.*, 2018), vitamin C content (Arel *et al.*, 2017), total carotenoid content (Rifqi *et al.*, 2023), and identification of active compounds by LC-MS (Abdullah *et al.*, 2017; Maharani *et al.*, 2016).

When applicable, the data were evaluated using analysis of variance (ANOVA), and differences among means were identified through appropriate post-hoc tests with a significance threshold of $p < 0.05$. All statistical calculations were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1 Red melinjo peel ethanolic extract

Phytochemical screening is an essential preliminary approach to identify the classes of bioactive compounds present in plant extracts. The presence and diversity of phytochemicals such as phenolics, flavonoids, alkaloids, saponins, and tannins are closely related to the biological activities of plant materials, including antimicrobial and antioxidant properties. Previous studies have shown that plant extracts rich in polar secondary metabolites tend to exhibit strong bioactivity, particularly when extracted using polar solvents such as ethanol (Agustina *et al.*, 2016; DepKes, 2000). Therefore, qualitative phytochemical analysis was conducted to provide an initial overview of the chemical constituents present in the red melinjo peel ethanolic extract and to support its observed biofunctional properties.

3.2 Qualitative analysis of phytochemical components

As seen in **Table 1**, Phytochemical analysis showed that the ethanol extract comprised glycosides, triterpenoids, alkaloids, saponins, phenolics, flavonoids, and tannins. The presence of these phytochemical components may be associated with the polar nature of the solvent (ethanol) used to obtain the red melinjo peel extract. During the extraction process, phytochemical components of plants will dissolve or bind with the solvent according to their polarity. The results are consistent with the principle that molecular similarity determines solubility: polar molecules dissolve readily in polar media, whereas non-polar molecules dissolve more effectively in non-polar media (Agustina *et al.*, 2016; DepKes, 2000; Rahmadita and Purwantisari, 2025).

Table 1. Qualitative analysis result of phytochemical components of red melinjo peel ethanolic extract

Phytochemical components	Analysis result
Alkaloids	+
Saponins	+
Tannins	+
Phenolics	+
Flavonoids	+
Triterpenoids	+
Steroids	-
Glycosides	+

Notes: +: Exist -: Did not exist

3.3 Antimicrobial activity test of red melinjo peel ethanolic extract

Plant-derived extracts have gained increasing attention as alternative antimicrobial agents due to the presence of bioactive secondary metabolites such as phenolics, flavonoids, tannins, and alkaloids. These compounds have been widely reported to interfere with microbial cell structures and metabolic processes, leading to growth inhibition or cell death. Previous studies have demonstrated that red melinjo (*Gnetum gnemon* L.) peel contains various phytochemicals with antimicrobial potential, particularly against foodborne pathogenic bacteria (Parhusip and Sitanggang, 2011; Angel and Parhusip, 2024). However, the antimicrobial effectiveness of plant extracts is highly dependent on factors such as extract concentration, type of microorganism, and differences in microbial cell wall structure. Therefore, by examining inhibition zones and comparing microbial sensitivity across different extract concentrations, this analysis aims to clarify the antibacterial spectrum of the extract and to identify an effective concentration that may be applied as a natural antimicrobial agent.

The results of the statistical analysis using univariate ANOVA showed a significant interaction ($p < 0.05$) between the concentration of red melinjo peel ethanolic extract and the microorganisms used in the antimicrobial activity test, in relation to the inhibition zone.

As shown in **Figure 1**, the negative control (0%), which was the ethanol used as the solvent to obtain the red melinjo peel extract, did not exhibit any inhibitory effect against the four test microorganisms. Therefore, the effect of ethanol could be disregarded.

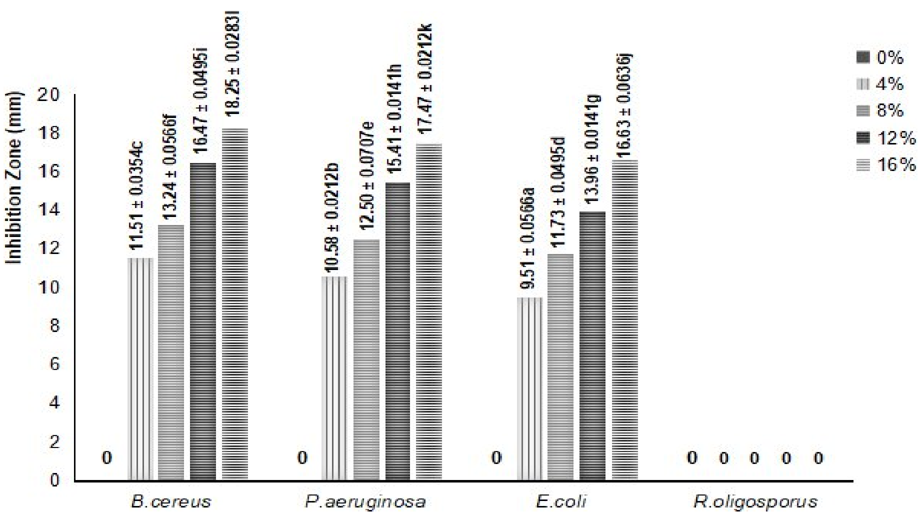

Figure 1. Inhibition zone or diameter of red melinjo peel ethanolic extract towards *B. cereus, P. aeruginosa, E. coli* and *R. oligosporus*

Note: Values with different superscript letters are significantly different ($p \leq 0.05$).

It was also shown that red melinjo peel ethanolic extract at concentrations of 4%, 8%, 12%, and 16% exhibited inhibitory activity against Gram-negative bacteria (*E. coli*, *P. aeruginosa*) and Gram-positive bacteria

(*B. cereus*); however, it did not show any inhibitory effect against *Rhizopus oligosporus*. Similar results were observed by Angel and Parhusip (2024), who found that the ethyl acetate extract of red melinjo peel effectively suppressed the growth of *E. coli*, *B. cereus*, and *P. aeruginosa*, yet did not inhibit the mold *R. oligosporus*. According to their study, the inability of the extract to inhibit *R. oligosporus* may be due to the absence of essential oils in red melinjo peel. Essential oils are known to interact with ergosterol—a sterol unique to fungal membranes—causing damage to the membrane and leading to leakage of intracellular components (Al-Shahrani *et al.*, 2017). In the absence of essential oil content, as suspected in red melinjo peel, such interaction and subsequent inhibition are unlikely to occur.

The inability of red melinjo peel ethanolic extract to inhibit the growth of *Rhizopus oligosporus* might be due to the extract concentration not being strong enough to suppress mold growth. *Rhizopus oligosporus* is a eukaryotic microorganism, possessing a more complex structure compared to prokaryotic microorganisms such as bacteria (Talaro and Chess, 2017). The cell membrane of eukaryotic microorganisms contains ergosterol, a sterol component that contributes to membrane strength and stability. Sterol compounds are known to prevent lysis caused by osmotic pressure (Al-Shahrani *et al.*, 2017; Tortora *et al.*, 2016).

As seen in **Figure 1**, the inhibition zones of *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Escherichia coli* increased with higher concentrations of red melinjo peel ethanolic extract. Overall, the average inhibition diameter of red melinjo peel ethanolic extract against the Gram-positive bacterium *B. cereus* (14.87 mm) was greater than that of the Gram-negative bacteria *P. aeruginosa* (13.99 mm) and *E. coli* (12.96 mm). The difference in inhibition zones between Gram-positive and Gram-negative bacteria indicates varying levels of sensitivity.

Variations in how Gram-positive and Gram-negative bacteria react to antimicrobial agents can be attributed to their cell wall architecture. Gram-positive cells are characterized by thick peptidoglycan reinforced by teichoic acids and limited lipid content, whereas Gram-negative cells have a two-membrane system: the inner membrane with peptidoglycan and an outer membrane that includes proteins, phospholipids, and lipopolysaccharides (Suprianto *et al.*, 2023).

The outer membrane found in Gram-negative bacteria but absent in Gram-positive bacteria makes it more difficult for antimicrobial agents to penetrate the peptidoglycan layer and reach the bacterial cell. This explains why the inhibition zone produced by the red melinjo peel ethanolic extract was larger for the Gram-positive *B. cereus* than for the Gram-negative *P. aeruginosa* and *E. coli* (Suprianto *et al.*, 2023).

According to Yanti and Mitika (2017), antibacterial compounds can be classified into four categories based on their ability to inhibit bacterial growth: weak (≤ 5 mm), mild (6–10 mm), strong (11–20 mm), and very strong (≥ 21 mm). Based on the inhibition zone size and overall effectiveness, a concentration of 12% red melinjo peel ethanolic extract was selected. As at this level, it can already be categorized as a strong antibacterial agent.

3.4 Evaluation of the minimum inhibitory and minimum bactericidal concentrations of ethanol-extracted red melinjo peel

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) is a crucial step in evaluating the antimicrobial effectiveness of plant extracts. These parameters provide insight into the lowest concentration required to inhibit microbial growth and to cause microbial cell death, respectively. Differences in MIC and MBC values among microorganisms are often influenced by variations in cell wall structure, membrane composition, and intrinsic resistance mechanisms (Owuama, 2017). By evaluating MIC and MBC values against representative Gram-positive and Gram-negative bacteria, the antibacterial potential and spectrum of activity of the red melinjo peel ethanolic extract can be more clearly understood.

As seen in **Table 2**, *B. cereus* (Gram-positive) required lower concentrations of the ethanolic extract to achieve MIC and MBC compared with the Gram-negative bacteria (*P. aeruginosa* and *E. coli*). In contrast, the Gram-negative strains displayed higher MIC and MBC values, indicating that a stronger concentration of the extract was needed to suppress or eliminate their growth (Owuama, 2017).

Table 2. MIC and MBC values of red melinjo peel ethanolic extract towards 3 bacteria

Microorganism	MIC (%)	MBC (%)
B. cereus	0.46	1.83
P. aeruginosa	0.50	2.01
E. coli	0.56	2.22

This variation arises from the contrasting cell wall architectures of the two bacterial groups. Gram-negative organisms feature an additional outer membrane composed of proteins, phospholipids, and lipopolysaccharides, which is not present in Gram-positive cells. This external layer restricts antimicrobial entry, making Gram-negative bacteria less susceptible, whereas Gram-positive bacteria with only a thick peptidoglycan layer are more readily inhibited (Suprianto *et al.*, 2023).

3.5 Antioxidant activity stability of red melinjo peel ethanolic extract upon storage time

Antioxidant activity is a critical functional property of plant extracts; however, it may change during storage due to the degradation of bioactive compounds. Factors such as temperature, oxygen exposure, and storage duration can significantly affect the stability of antioxidant constituents, resulting in decreased radical scavenging capacity over time (Dewi *et al.*, 2022). Evaluating antioxidant stability during storage is therefore necessary to assess the potential shelf life and practical applicability of the extract. In this study, antioxidant activity was monitored using the IC_{50} parameter to determine how storage time influences the radical scavenging ability of red melinjo peel ethanolic extract.

Figure 2 shows that storage caused the IC_{50} of the red melinjo peel ethanolic extract to increase. Because IC_{50} measures how much extract is needed to neutralize 50% of DPPH radicals, a smaller value reflects better antioxidant efficiency (Haryani, 2016; Tristantini *et al.*, 2016). Therefore, the extract displayed its greatest antioxidant strength on day 0, after which its ability steadily weakened over time.

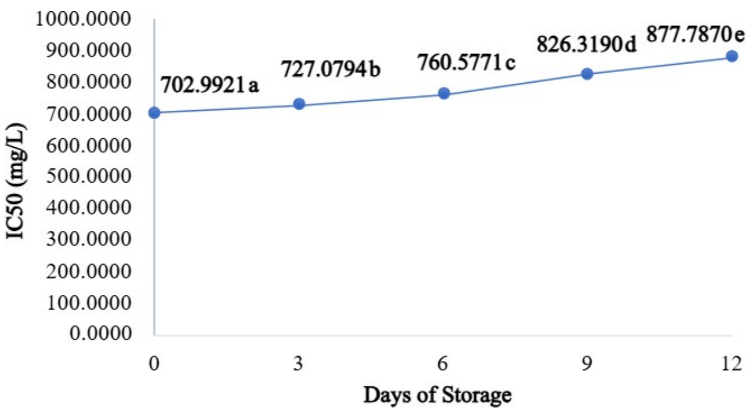

Figure 2. IC_{50} (mg/L) values of red melinjo peel ethanolic extract upon 12 days of storage time
Note: Values with different superscript letters are significantly different ($p \leq 0.05$).

A similar reduction in antioxidant performance during storage was also reported by Qu *et al.* (2012) in strawberry products fortified with encapsulated pomegranate peel extract stored for 90 days at 25°C. Antioxidant activity decreased with time, and this reduction was associated with the loss of polyphenolic components.

A decrease in antioxidant capacity could be linked to the gradual degradation of bioactive antioxidant constituents while the extract is stored at low temperatures (Dewi *et al.*, 2022). Components that contribute to antioxidant activity in the extract include phenolics, flavonoids, vitamin C, and carotenoids. A substance rich in these compounds typically shows strong antioxidant activity (Adawiah *et al.*, 2015; Ibrahim *et al.*, 2024).

3.6 Total phenolic test of red melinjo peel ethanolic extract upon storage time

Phenolic compounds are widely recognized as major contributors to the antioxidant activity of plant extracts due to their ability to donate hydrogen atoms and neutralize free radicals. Numerous studies have demonstrated a strong positive correlation between total phenolic content and antioxidant capacity in plant-based materials (Adawiah *et al.*, 2015). However, phenolic compounds are also susceptible to degradation during storage as a result of oxidation, light exposure, and temperature fluctuations. Therefore, measuring changes in total phenolic content over storage time is essential to understand its role in the observed decline in antioxidant activity of the red melinjo peel ethanolic extract.

As illustrated in **Figure 3**, the total phenolic content of the extract declined over storage time. Furthermore, a correlation test shown in **Figure 4** revealed that the decrease in total phenolic content was associated with the decline in antioxidant activity over 12 days of storage. This supports the findings of Adawiah *et al.* (2015), who stated that high antioxidant activity in a sample is strongly correlated with its phenolic content.

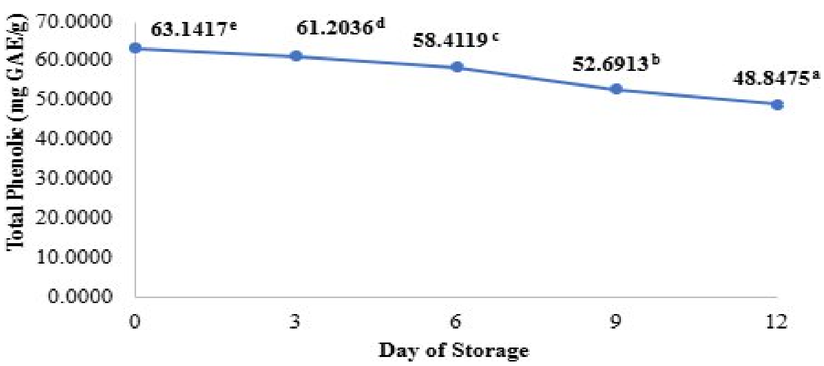

Figure 3. Total phenolic (mg GAE/g) of red melinjo peel ethanolic extract upon 12 days of storage time
Note: Values with different superscript letters are significantly different ($p \leq 0.05$).

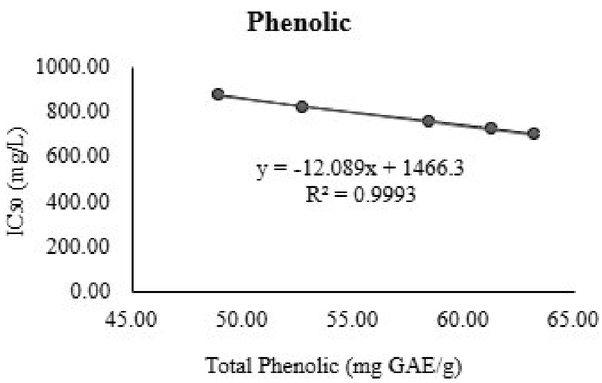

Figure 4. Correlation test of total phenolic value towards antioxidant activity

The result of the research also aligns with the findings of Qu *et al.* (2012), which stated that the antioxidant activity of pomegranate peel extract decreased over storage time, correlating with the reduction in polyphenol content. Phenolic compounds contribute to antioxidant activity because their hydroxyl groups can release hydrogen atoms, converting free radicals (•RO) into more stable forms (ROH) (Adawiah *et al.*, 2015; Ibrahim *et al.*, 2024).

The decline in total phenolic content of the red melinjo peel ethanolic extract may be caused by oxidation of phenolic compounds in the presence of oxygen during storage. Besides oxygen, other factors that can influence the degradation include storage temperature, light, and pH (Ali *et al.*, 2018; Tristanto *et al.*, 2017; Wisnu *et al.*, 2015).

3.7 Total flavonoid test of red melinjo peel ethanolic extract upon storage time

Flavonoids represent an important subgroup of phenolic compounds and play a significant role in antioxidant defense mechanisms by scavenging reactive oxygen species and stabilizing free radicals. High flavonoid content is often associated with enhanced antioxidant performance in plant extracts (Ibrahim *et al.*, 2024). Like phenolics, flavonoids are chemically unstable and may degrade during storage due to oxidative processes and environmental factors. Thus, evaluation of total flavonoid content during storage is necessary to clarify its contribution to antioxidant activity changes and to complement the analysis of total phenolic content.

As shown in **Figure 5**, the total flavonoid content of the red melinjo peel ethanolic extract decreased with increasing storage time. Moreover, the correlation test results presented in **Figure 6** indicate that the decline in flavonoid content was associated with the reduction in antioxidant activity over the 12-day storage period. This supports the findings of Adawiah *et al.* (2015), which noted that high antioxidant activity is strongly associated with the flavonoid content of the sample.

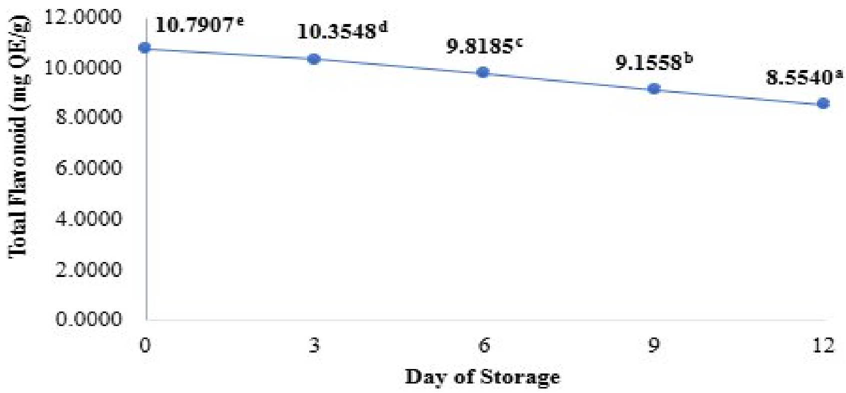

Figure 5. Total flavonoid (mg QE/g) of red melinjo peel ethanolic extract upon 12 days of storage time
Note: Values with different superscript letters are significantly different ($p \leq 0.05$).

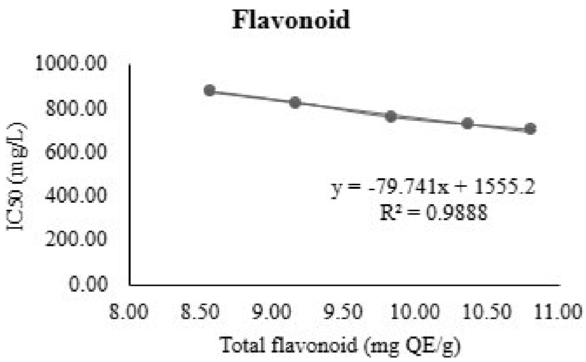

Figure 6. Correlation test of total flavonoid value towards antioxidant activity

Flavonoids possess antioxidant properties as they can donate hydrogen atoms to stabilize free radicals and also scavenge reactive oxygen species (ROS). Total flavonoid content is closely related to total phenolic content since flavonoids are a subclass of phenolic compounds. Therefore, a high phenolic content generally implies a high flavonoid content (Adawiah *et al.*, 2015; Ibrahim *et al.*, 2024).

The decrease in total flavonoid content may be due to the oxidation of flavonoid compounds by oxygen during storage. Oxidation can alter the chemical structure, leading to a reduction or loss of antioxidant activity. Besides oxygen, storage temperature and exposure to light can also contribute to this degradation (Dewi *et al.*, 2022; Ferdinal *et al.*, 2013).

3.8 Total vitamin C test of red melinjo peel ethanolic extract upon storage time

Vitamin C (ascorbic acid) is a water-soluble antioxidant that plays a crucial role in scavenging free radicals and protecting biological systems from oxidative stress. In plant-based extracts, vitamin C contributes significantly to overall antioxidant activity, particularly through its ability to donate electrons and regenerate other antioxidant compounds. However, vitamin C is chemically unstable and highly susceptible to degradation during storage due to oxidation, temperature fluctuations, light exposure, and pH conditions. Therefore, evaluating changes in vitamin C content during storage is essential to understand its contribution to the antioxidant stability of red melinjo peel ethanolic extract.

As shown in Figure 7, the total vitamin C content declined over the storage period. Furthermore, the correlation test in Figure 8 demonstrates a relationship between the reduction in vitamin C content and the decrease in antioxidant activity of the red melinjo peel ethanolic extract during the 12-day storage period. This supports the findings of Adawiah *et al.* (2015), which reported that antioxidant activity is closely linked to vitamin C content.

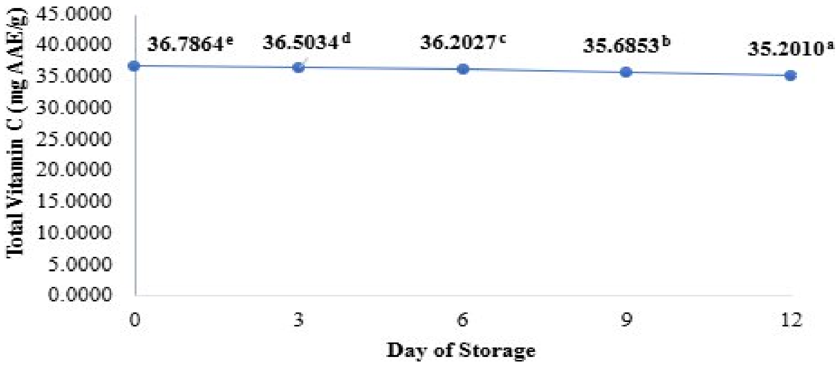

Figure 7. Total vitamin C (mg AAE/g) of red melinjo peel ethanolic extract upon 12 days of storage time
Note: Values with different superscript letters are significantly different ($p \leq 0.05$).

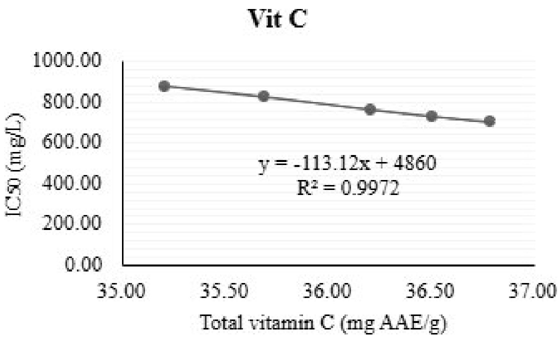

Figure 8. Correlation test of total vitamin C value towards antioxidant activity

Vitamin C (ascorbic acid) exhibits antioxidant activity by donating electrons in both intracellular and extracellular biochemical reactions, thereby neutralizing reactive oxygen species (Adawiah *et al.*, 2015; Ibrahim *et al.*, 2024). The degradation of vitamin C content can occur due to oxidation by oxygen, and is also influenced by storage temperature, light, and pH (Ibrahim *et al.*, 2024).

3.9 Total carotenoid test of red melinjo peel ethanolic extract upon storage time

Carotenoids are lipid-soluble pigments widely recognized for their antioxidant properties and their role in neutralizing reactive oxygen species. In addition to their health-promoting effects, carotenoids contribute to the characteristic red and orange coloration of many plant materials, including red melinjo peel. Despite their functional importance, carotenoids are prone to oxidative degradation during storage, particularly in the presence of oxygen and elevated temperatures. Monitoring total carotenoid content over storage time is therefore necessary to assess its influence on antioxidant activity and the potential application of red melinjo peel extract as a functional ingredient.

As illustrated in Figure 9, total carotenoid content declined over time. The correlation analysis in Figure 10 confirms that the reduction in carotenoid content was associated with decreased antioxidant activity of the extract during the 12-day storage period. This observation is in line with Manasika and Widjanarko (2015), who found that the decrease in total carotenoids was directly linked to declining antioxidant activity.

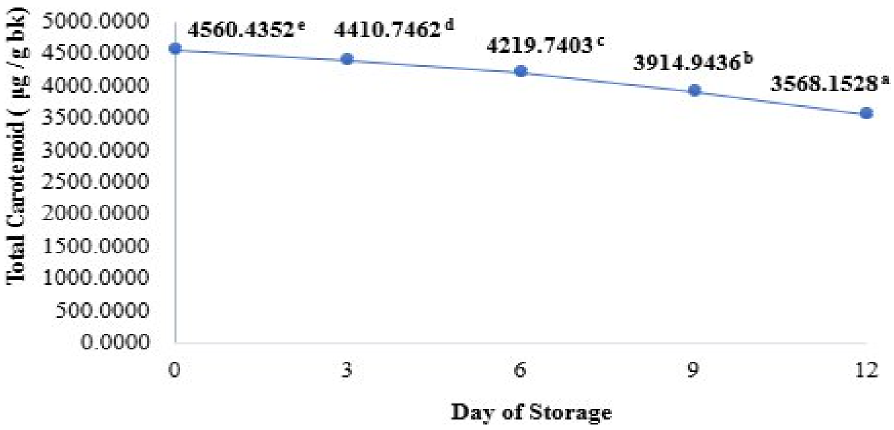

Figure 9. Total carotenoid (μg/g) of red melinjo peel ethanolic extract upon 12 days of storage time
Note: Values with different superscript letters are significantly different ($p \leq 0.05$).

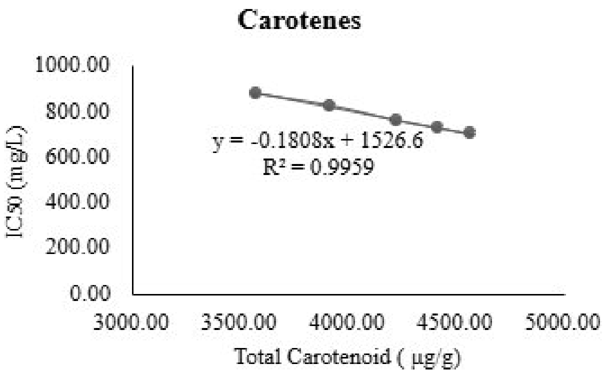

Figure 10. Correlation test of total carotenoid value towards antioxidant activity

Carotenoids can stabilize free radicals by donating electrons (Adawiah *et al.*, 2015; Ibrahim *et al.*, 2024). The reduction in carotenoid content of the extract may result from oxidation of β-carotene pigments in the presence of oxygen. Oxidation damages the chromophore group in β-carotene, leading to pigment fading. Additionally, carotenoid stability can be affected by storage temperature (Oktora *et al.*, 2016).

Carotenoids from the red melinjo peel ethanolic extract can also be utilized as natural colorants in food products, as they are pigments that produce orange, yellow, and red hues. Their function as antioxidants contributes to health benefits, making the extract a functional and aesthetic additive in food products (Maleta *et al.*, 2018; Ibrahim *et al.*, 2024).

3.10 Active Compounds 1analysis of red melinjo peel ethanolic extract through Liquid Chromatography–Mass Spectrometry (LC-MS)

The biological activity of plant extracts is determined not only by the total amount of bioactive compounds present but also by the diversity and chemical nature of individual constituents. Advanced analytical techniques such as liquid chromatography–mass spectrometry (LC-MS) enable the identification of specific active compounds responsible for antimicrobial and antioxidant effects. Characterizing these compounds is essential for understanding the mechanisms underlying the bioactivity of red melinjo peel ethanolic extract and for supporting its potential application in food, pharmaceutical, or nutraceutical products. Therefore, LC-MS analysis was performed to identify the active compounds contributing to the observed antibacterial and antioxidant properties of the extract.

Based on the LC-MS analysis, a total of 16 active compounds were identified in the red melinjo peel ethanolic extract that contribute to its antibacterial and antioxidant properties. Among these, 12 compounds identified are methyl nicotinate (trigonelline); 4-[28-oxo-28-{[2-(2-pyridinyl)ethyl]amino}lupa-2,20(29)-dien-3-yl]benzoic acid; valinol; indoline; linaroside; 8-methyl-3-[{2-methyl-1-[1-(2-methyl-2-butanyl)-1H-tetrazol-5-yl]propyl}[2-(4-morpholinyl)ethyl]amino)methyl]-2(1H)-quinolinone; piperidine; 5-(1-piperidinylmethyl)-1-benzofuran-2-carboxamide; 2-methoxybenzaldehyde; lupenone; N-(3-methoxy-5-nitrophenyl)-2-(5-methyl-3,4-dinitro-1H-pyrazol-1-yl)acetamide. Meanwhile, four other compounds were found to possess only antibacterial activity, namely amino nonanoic acid, pyrroline (or dihydropyrrole), acetic acid 3-(4-methoxy-phenoxy)-4-oxo-4H-chromen-7-yl ester, and acetic acid, 2-[[3-(2,5-dimethoxybenzoyl)-5-benzofuranyl] oxy]. These findings suggest that the red melinjo peel ethanolic extract contains a diverse range of bioactive compounds that may synergistically contribute to its observed antibacterial and antioxidant efficacy.

4. CONCLUSIONS

Qualitative phytochemical screening confirmed that the red melinjo (*Gnetum gnemon* L.) peel ethanolic extract contains multiple bioactive classes, including saponins, tannins, flavonoids, glycosides, phenolics, triterpenoids, alkaloids. The extract exhibited antibacterial activity against both Gram-positive (*Bacillus cereus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), but no inhibitory effect was observed against the mold *Rhizopus oligosporus*. The MIC values for *B. cereus*, *P. aeruginosa*, and *E. coli* were 0.46%, 0.50%, and 0.56%, respectively, while the MBC values were 1.83%, 2.01%, and 2.22%. Based on inhibition performance and practical efficiency, the 12% extract concentration was selected for subsequent analyses.

The extract also showed antioxidant activity, with an IC_{50} value of 702.9921 mg/L at day 0. Nevertheless, antioxidant capacity decreased progressively during 12 days of low-temperature storage. This decline was consistent with reductions in key antioxidant-related components, namely total phenolics, total flavonoids, vitamin C, and carotenoids, indicating that storage time contributes to the degradation of multiple antioxidant contributors and reduces overall extract functionality.

LC–MS profiling identified 16 active compounds in the extract. Twelve compounds were associated with both antibacterial and antioxidant activities, while four compounds were linked to antibacterial activity only. Overall, these results indicate that red melinjo peel ethanolic extract is a promising bioactive material with dual functionality.

This study provides novelty by integrating (i) antibacterial evaluation across Gram-positive, Gram-negative bacteria, and mold models, (ii) MIC/MBC determination, (iii) storage-dependent antioxidant stability assessment linked to quantitative changes in major antioxidant components, and (iv) compound-level characterization via LC–MS within a single research framework for red melinjo peel ethanolic extract.

Several limitations should be noted. The study did not evaluate the effects of thermal processing, light exposure, oxygen control, or pH variation on extract stability, all of which are relevant to real food systems. In addition, antimicrobial performance was assessed mainly through in vitro assays and was not validated in a food model system where matrix interactions may reduce activity.

Future research should focus on improving stability and application feasibility using food technology approaches, such as nanoencapsulation or microencapsulation to protect phenolics, vitamin C, and carotenoids during storage; incorporation into edible coatings or active packaging films; and validation in real food matrices (food model systems) to assess efficacy under practical conditions. Further studies may also examine the kinetics of degradation under different temperatures and pH conditions, as well as sensory and safety aspects to support potential industrial utilization.

5. SUGGESTIONS

Further research should be conducted to evaluate the stability of red melinjo peel ethanolic extract under varying conditions such as pH levels, sugar and salt concentrations, and different heating durations and temperatures. This is important to assess its feasibility for applications in the food industry. Additionally, in vivo toxicity studies are necessary to determine its safety for consumption and use in living organisms.

Given it demonstrated antibacterial and antioxidant properties, along with its carotenoid content, the red melinjo peel ethanolic extract holds promise as a natural food additive. Its application is particularly suitable for food products that do not undergo heat processing, such as yogurt, to preserve its bioactive and functional properties, especially its carotenoid stability and overall effectiveness.

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